Transport and Ecotoxicity of C_{60} Fullerenes in the Terrestrial Environment

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By

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ABSTRACT

With uses ranging from ingredients in sunscreen and tires to applications in soil remediation and organ specific drug delivery, the use of engineered nanomaterials is becoming increasingly prevalent in modern society. Unfortunately, very little is known about the transport of these materials in soil or the resulting ecotoxicity and only a handful of studies have been done in this area. Soil column tests were performed to assess the affect of pH on the transport of these particles through porous medium. Using a commercially available sand (Accusand) as the porous medium tests were conducted at pH 5 and pH 9. Results indicated that a difference in particle surface charge at the respective pH levels greatly impacted transport of the fullerenes. No breakthrough was observed at pH 5 while approximately 60% breakthrough occurred at pH 9. Reproduction tests were performed on enchytraeid worms (*Enchytraeus crypticus*) to assess ecotoxicity. Tests were conducted using fullerene concentrations of 1% and 0.1% by weight in a sandy soil. Results indicated that fullerenes have no impact on *Enchytraeus crypticus* reproduction.

ACKNOWLEDGMENTS

There are many people whose help was instrumental in the completion of this work. First and foremost I would like to express thanks to my advisor, Dr. John Lenhart who good-naturedly disregarded my impatience and me constantly stopping by his office without appointment for advice. Dr. Harold Walker must be thanked for proposing that I conduct this research and giving advice when Dr. Lenhart was unavailable. I am also grateful to Dr. Roman Lanno for helping me understand the ecotoxicity portion of this research.

I am indebted to Yu Sik Hwang for helping me understand the use of various instruments and staying in the lab with me until late hours in order to understand why experiments were not performing as expected. Yu Sik also independently fit the breakthrough curve for KBr spending his own time on this for which I must express my thanks. Similarly, Dr. Ziqi He often sacrificed his time and helped with the TOC and anion chromatography analysis for which I am thankful. Finally, I must thank Shuo Yu. Without her help I would not have been able to conduct the *Enchytraeus crypticus* reproduction tests.

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CHAPTER 1

INTRODUCTION

Projected to become the foundation for a trillion dollar industry, nanoparticles (nominally 1 to 100 nm in diameter) have special chemical properties which account for their vast potential. Figure 1 shows how this market is projected to grow by the year 2015. Figure 2 indicates fullerenes share of this market according to number of U.S. patents granted from 2000 until 2006. Their extremely small size and quantum effects provide a large, highly reactive surface area available for chemical bonding. It is largely unknown whether these same properties that cause engineers and scientists to be so excited about the possibilities of nanomaterials may lead to a greater environmental risk. The transport and accumulation of colloidal particles (from 10 nm to 1 μm) in soil has been extensively studied (e.g., Lenhart and Saiers 2003). However, little is known about the transport and accumulation of nanomaterials in soil. Many factors affect the transport of ultrafine particles in soil including pH, redox potential, water velocity, pore size, organic composition of soil, and soil saturation, to name a few (McCarthy and Zachara 1989). All of these factors need to be studied individually to understand nanoparticle fate and transport in the terrestrial environment.

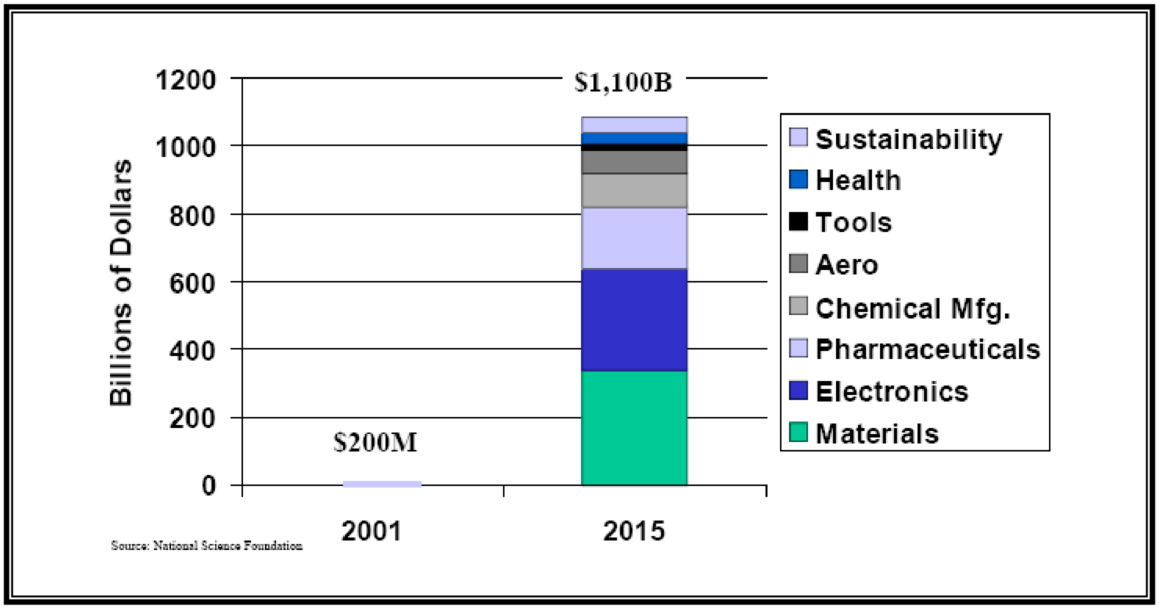

Figure 1 – Projected growth of the nanotechnology market over the period from 2001 through 2015. Taken from Tran (2007)

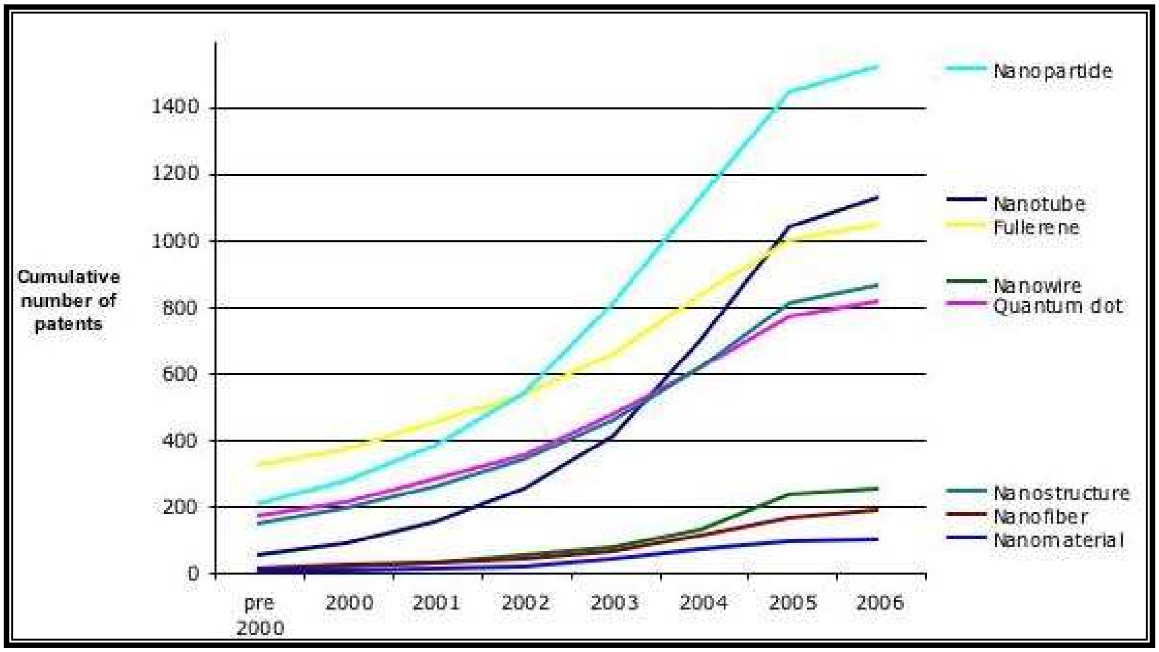

Figure 2 – Growth of various nanotechnology patents from 2000 until 2006. Note the higher growth of the fullerene patents in relation to other segments. Taken from Tran (2007)

The unique features of nanoparticles may lead to greater risk of bioavailability (Wiesner 2003). The increased reactivity of nanoparticles affects their potential for aggregation and deposition (Lecoanet and Wiesner 2004) which may in turn increase their toxicity in soil. At this time insufficient studies have been done to quantify the effects of nanomaterials in soil over time. Preliminary studies have shown that due to the varying surface chemistries of different nanomaterials, transport and accumulation may vary from one type of nanoparticle to another (Lecoanet et al. 2004).

Due to the complex nature of studying this problem and the incomplete nature of published work, it is necessary to research each type of nanoparticle and the effect of different variables individually. For the purpose of this research, the effect of pH on C_{60} fullerene transport in soil was studied in columns of sand (Lenhart and Saiers 2003, Lecoanet and Wiesner 2004). Aside from information necessary to ascertain transport, accumulation, and bioavailability further research may be useful in other areas of engineering such as soil remediation and water purification.

Soil accumulation and bioavailability data is only valuable when compared to biological effects (Colvin 2003). While a few studies have been done to assess the toxicity of fullerenes on humans and mice (Colvin 2003) relatively few studies have been done on other species to evaluate ecotoxicity. Oberdörster (2004) found that fullerenes caused damage to the cells of fish brains. Lovern and Klaper (2006) found that fullerene concentrations of 880 parts per billion caused total mortality over 48 hours in water fleas (*Daphnia magna*). Literature reviews failed to find any information regarding the toxicity of fullerenes to soil insects. Since earthworms are excellent indicators of the health of a soil ecosystem it makes sense to evaluate whether reasonable soil concentrations cause statistically relevant mortality or reproductive problems in earthworm populations. To this end reproduction tests were performed on enchytraeid worms (*Enchytraeus crypticus*) to evaluate the ecotoxicity of fullerenes in soil.

There were two main objectives of this research. The first was to evaluate how pH affects the transport of C_{60} fullerenes in quartz sand and use this data to arrive at a worst case scenario for reasonable soil accumulations. Secondly, the research was to focus on whether reasonable soil accumulations are statistically relevant in regards to ecotoxicity through reproduction studies using enchytraeid worms (*Enchytraeus crypticus*).

CHAPER 2

EXPERIMENTAL METHODS

2.1 Overview

The research was conducted in two phases: transport of fullerenes through a quartz sand medium at an ionic strength of 0.01 M and two different pH levels (pH 5 and pH 9), and reproduction tests performed on enchytraeid worms (*Enchytraeus crypticus*) in order to assess ecotoxicity. These two phases were conducted individually with the final data compared in order to draw conclusions. C_{60} fullerenes of 99.5+% purity were purchased from SES Research, Houston, TX and were used as received. *Enchytraeus crypticus* specimens were provided courtesy of Dr. Lanno and the entomology department at The Ohio State University.

The C_{60} fullerenes were characterized in order to better evaluate particle transport and ecotoxicity. A particle size analyzer (90Plus, Brookhaven Instruments Corp., NY) was used to determine average size and size distribution of the C_{60} fullerene aggregates. The fullerene concentrations in column effluent samples were calculated using a UV/vis spectrophotometer (Shimadzu UV-2401 PC). The concentration of fullerenes in a prepared stock suspension was measured by a total organic carbon analyzer (Shimadzu TOC-500A). Knowing the concentration in the stock solution enabled a comparison of UV absorbance to carbon concentration.

Reproduction tests were conducted using two different soil concentrations and a reference soil. Webster soil was used for the reference soil since it has been well studied in regards to *Enchytraeus crypticus* reproduction. The fullerenes were added to Sassafras soil because it is a sandy soil. Column experiments were conducted using quartz sand; therefore, the fullerene concentrations maintained within the column could be compared more readily to a sandy soil to ascertain whether C_{60} fullerenes may have any ecotoxic effects in regards to *Enchytraeus crypticus* reproduction.

2.2 Preparation of Materials for Column Experiments

Quartz sand purchased from Unimin Corp (Accusand 50/70) was washed in Millipore water and sifted using a 150-μm stainless steel sieve until the rinsing solution appeared particle-free. This sand was used as the porous medium in all of the column experiments. Accusand characterization of several different grades as compiled by Schroth et al. (1996) is shown below in Table 1.

	12/20	20/30	30/40	40/50
		Physical properties		
Particle diameter d_{50} (mm)	1.105 ± 0.014†	0.713 ± 0.023	0.532 ± 0.003	0.359 ± 0.010
Uniformity coefficient d_{60}/d_{10}	1.231 ± 0.043	1.190 ± 0.028	1.207 ± 0.008	1.200 ± 0.018
Particle sphericity	0.9	0.9	0.9	0.9
Particle density, Mg/m³	2.665	2.664	2.665	2.663
		Chemical analyses		
Cation-exchange capacity, $cmol_c$/kg	0.60	0.57	0.62	0.67
Total Fe, g/kg	9.31	7.64	7.65	5.58
Fe oxides, g/kg	0.36	0.29	0.34	0.30
Organic C, g/kg	0.3	0.4	0.3	0.3
Total Cd, mg/kg	<7.0	<7.0	<7.0	<7.0
Total Cu, mg/kg	<14.0	<14.0	<14.0	<14.0
Total Pb, mg/kg	<5.0	<5.0	<5.0	<5.0
Total Mn, mg/kg	51.6	43.6	40.3	34.1
Total Zn, mg/kg	9.95	6.98	7.1	6.18

† Averages with standard deviations. Total number of sieve analyses were 19 for 12/20, 170 for 20/30, 9 for 30/40, and 4 for 40/50 sand.

Table 1 - Physical properties and chemical analyses for four Accusand grades. Table taken from Schroth et al. (1996)

Following the method described in Brant et al. (2006), 80 mg of C_{60} fullerenes were mixed with 100 ml of Millipore water for approximately 10 days using a magnetic bar stirrer. After stirring, the suspension was filtered through a 25-mm diameter Millipore 0.45 μm pore size HATF triton free, mixed cellulose fiber membrane using a Swinnex® filter holder attached to a syringe. The filter membrane was replaced after every 20 ml of suspension filtered. Filtering was conducted in order to obtain a more homogeneous suspension of fullerene particles.

An electrolyte solution that had been adjusted to the appropriate pH at an ionic strength of 0.01 M was prepared for each column experiment. The pH 5 electrolyte solution was prepared by adding 10 mL of 1 M NaCl solution and 100 μL of 0.1 M HCl to a 1-L volumetric flask then filling to the 1 L mark with Millipore water. The pH 9 electrolyte solution was prepared by adding 5 mL of 1 M $NaHCO_3$ and 5 mL of 1 M NaCl to a 1-L volumetric flask and then filling to the 1 L mark with Millipore water. Since atmospheric CO_2 will lower the pH, a few drops of 1 M NaOH were added to raise the pH as appropriate. Suspension pH was then tested after each preparation to ensure reliability.

A fullerene suspension that was adjusted to an ionic strength of 0.01 M and the appropriate pH was prepared immediately before performing each column experiment. For the pH 5 suspension, 700 μL of 0.01 M HCl and 1 mL of 1 M NaCl were added to a 100-mL volumetric flask and then the filtered fullerene suspension discussed above was added up to the 100 mL mark. For the pH 9 suspension, 0.5 mL of 1 M $NaHCO_3$ and 0.5 mL of 1 M NaCl were added to a 100 mL volumetric flask and then the filtered fullerene suspension was added up to the 100 mL mark.

The sand media was equilibrated to the appropriate pH by mixing 200 g of the prepared quartz sand in 1 L of the electrolyte solution overnight on a shaker table (New Brunswick Scientific C1 Platform Shaker). For the pH 5 solution it was necessary to repeat this process several times. The addition of several hundred μL of 0.1 M HCl was necessary to obtain an equilibrium condition of pH 5

2.3 Column Design

Transport experiments were performed to evaluate the transport and accumulation of fullerenes in soil. Column transport experiments were conducted using quartz sand packed into glass chromatography (Kontes) columns with a 2.5 cm internal diameter and 15 cm length based on procedures described by Lenhart and Saiers (2003). PTFE fittings and 20 μm porosity polyethylene bed supports were used in the columns. A syringe pump (Harvard Apparatus model 55-1144) with a 100 ml stainless-steel syringe was used to control the flow of fullerene suspension through the column. Alltech 1/8 inch inner diameter PTFE tubing connected the pump syringe to the column influent port at the bottom of the column with a ball valve switching between pump filling and infusing operations. Alltech 1/16 inch inner diameter PTFE tubing connected the column effluent port at the top of the column to a fraction collector. The column experiment layout is shown in Figure 3.

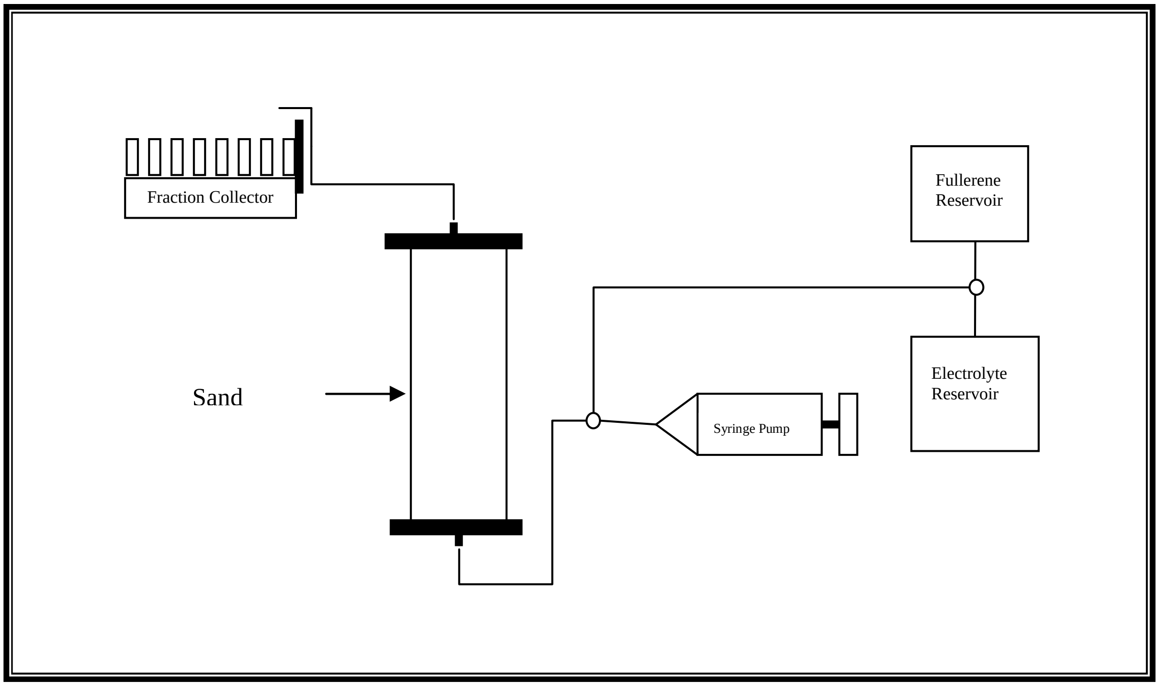

Figure 3 - Diagram of column experiment

Each column experiment was prepared by using the syringe pump to infuse an empty column with the appropriate electrolyte solution. A stainless steel stir rod was then used to assure no air bubbles were present on the lower bed support. At this time a 2-cm layer of wet sand that had been equilibrated to the appropriate pH, as discussed above, was added. The sand was then mixed with the stir rod to assure the removal of any entrained air bubbles. Layers were added and mixed together to ensure uniformity until a depth of 10 cm was obtained. When filled to the predetermined 10-cm line marked on the column, the column was vigorously tapped to allow settling of the sand. Additional sand was added and stirred until the sand settled to the line marked on the column. Approximately 89 g of dry sand was used to pack each column.

2.4 Column Experiment Methodology

Two hundred mL (approximately 10 pore volumes) of the electrolyte solution was infused through the column prior to running each column experiment. Effluent from the column was then checked for UV absorbance and pH to ensure that these parameters were identical to those of the influent electrolyte solution. When equilibrium conditions of the column were reached, 76 mL (approximately 4 pore volumes) of the pH adjusted fullerene suspension was infused through the column at a rate of 240 mL per hour. A fraction collector (Teledyne Isco Retriever 500) was used to collect samples at 1-minute intervals. After 19 minutes, the pump and fraction collector were stopped and the pump emptied of any remaining fullerene suspension. At this point the pump syringe was refilled with pH adjusted electrolyte solution. This solution was then infused through the column at a rate of 240 mL per hour for 11 minutes with samples gathered using the fraction collector at 1-minute intervals.

Fullerene concentrations of the effluent samples were determined by measuring the absorbance of light at 350 nm using a UV/vis spectrophotometer (Shimadzu UV-2401 PC). Fullerene concentration in the stock suspension was found to be 1.436 mg/L yielding an absorbance of 0.013. One column experiment was conducted using a 100 mg/L KBr solution as Br with 0.01 M NaCl instead of the fullerene influent. The Br concentration of the effluent was measured using anion chromatography (Dionex AS40 Automated Sampler, LC20 Chromatography Enclosure, ED40 Electrochemical Detector, GP50 Gradient Pump). This breakthrough curve was used to compare the fullerene suspension breakthrough with that of an inert tracer. Using STANMOD (version 2.2) – CXTFIT2 (Simunek et al. 1999) computer software the KBr breakthrough curve was fit to assess column properties.

2.5 Preparation of Materials for Reproduction Tests

Soil samples (20 g) were weighed and placed into 0.3-L canning jars. It was assumed that a 1% soil concentration of C_{60} fullerenes is at the high end of what could be reasonably found in the environment. Because of this, soil concentrations of 1% and 0.1% were tested. The reproduction tests were conducted in sets of 4 with one set containing Webster soil as the reference soil, one set containing Sassafras soil with a fullerene concentration of 20 mg C_{60}, and the final set containing Sassafras soil with a fullerene concentration of 200 mg C_{60}. Characterization of the various soil types is shown below in Table 2. For the Sassafras soil samples, 1 g of deionized water was placed in each jar and mixed until the water was evenly distributed throughout the sample. At this point the appropriate mass of fullerenes was added to the jar and mixed for several minutes until it was reasonable to assume the fullerenes were evenly distributed throughout the soil. After mixing in the fullerenes, 3 g of deionized water was added to each Sassafras soil sample. For the Webster soil samples 4 g of deionized water was added to each jar. All twelve jars were covered with a screw cap that had a hole punched in its center to allow for air circulation. The jars were weighed and labeled then placed in a refrigerator at 22 °C and left overnight.

Soil Type	Organic carbon %	pH	clay %
Webster	2.4	5.5	35.6
Sassafras	0.41	4.4	11.6

Table 2 – Characterization of Webster and Sassafras soils (Lanno 2008)

2.6 Reproduction tests methodology

After allowing the jars to equilibrate to test conditions, 10 adult *Enchytraeus crypticus* worms were placed in each jar along with 50 μg of ground Quaker Oats. The jars were weighed and if different from the weight recorded on the jar then deionized water was added as appropriate. The jars were then replaced in the refrigerator. Twice a week the worms were fed 50 μg of ground Quaker Oats and weighed with deionized water added as appropriate. After two weeks the adult worms were removed from each jar. The number of adult worms removed from each jar was recorded with a 20% mortality rate invalidating the test. Once again the worms were fed 50 μg of ground Quaker Oats. The samples were weighed at each feeding with deionized water added as appropriate. At the end of one month the newly hatched worms were counted using the Ludox floating method. This was done by mixing 20 mL of Ludox® AM-30 colloidal silica, 30% by weight suspended in water, with the soil in each test vessel and letting it sit for approximately 20 minutes. Juvenile worms would then float in the suspension and could be counted. It should be noted that the worms were extremely small and numerous requiring some estimation techniques. However, any error would be repeated throughout each sample neutralizing its effect.

CHAPTER 3

RESULTS AND DISCUSSION

3.1 Suspension Synthesis and Characterization

A fullerene suspension was prepared using the magnetic mixing method described by Brant et al. (2006). Particles were mixed for approximately 10 days until the suspension became dark brown in color. The suspension was then filtered through a 0.45 μm filter. After filtration the suspension was a very light, auburn color.

The best method of suspension preparation was discovered through trial and error. When trying to prepare suspensions in 500-mL batches, many problems were encountered. Vacuum filtration using 47 mm diameter filter membranes experienced clogging due to the larger mass of fullerenes in suspension. Teflon syringe filters were also tried with the result of all the fullerene particles being filtered out of suspension. After several attempts it was decided that preparing the suspensions in 100-mL batches as described above was the best method.

By measuring the UV absorbance and particle sizes over the next month suspension stability was ascertained. Size data gathered over this period is included in the appendix. The UV absorbance remained consistent while the effective diameter of the fullerene aggregates remained at approximately 202 nm (Figure 4). Particle size did not change with time. While there was a small variation in effective diameter, Figure 4 clearly shows that particle size remained approximately 202 nm. UV absorbance was a good indicator of fullerene concentration since absorbance will vary linearly with concentration. A typical UV absorbance plot for C_{60} fullerenes is shown below in Figure 5. The UV absorbance plots obtained were almost identical to work published by Fortner et al. (2005).

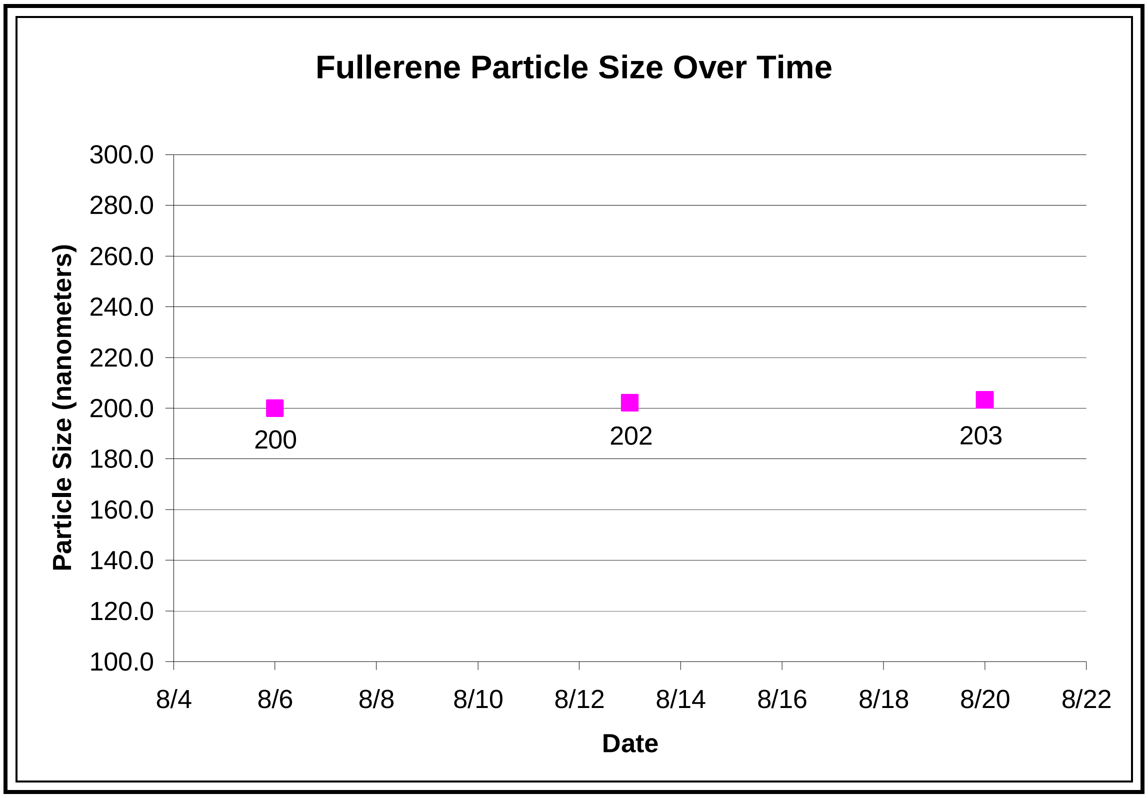

Figure 4 – C_{60} particle size over time

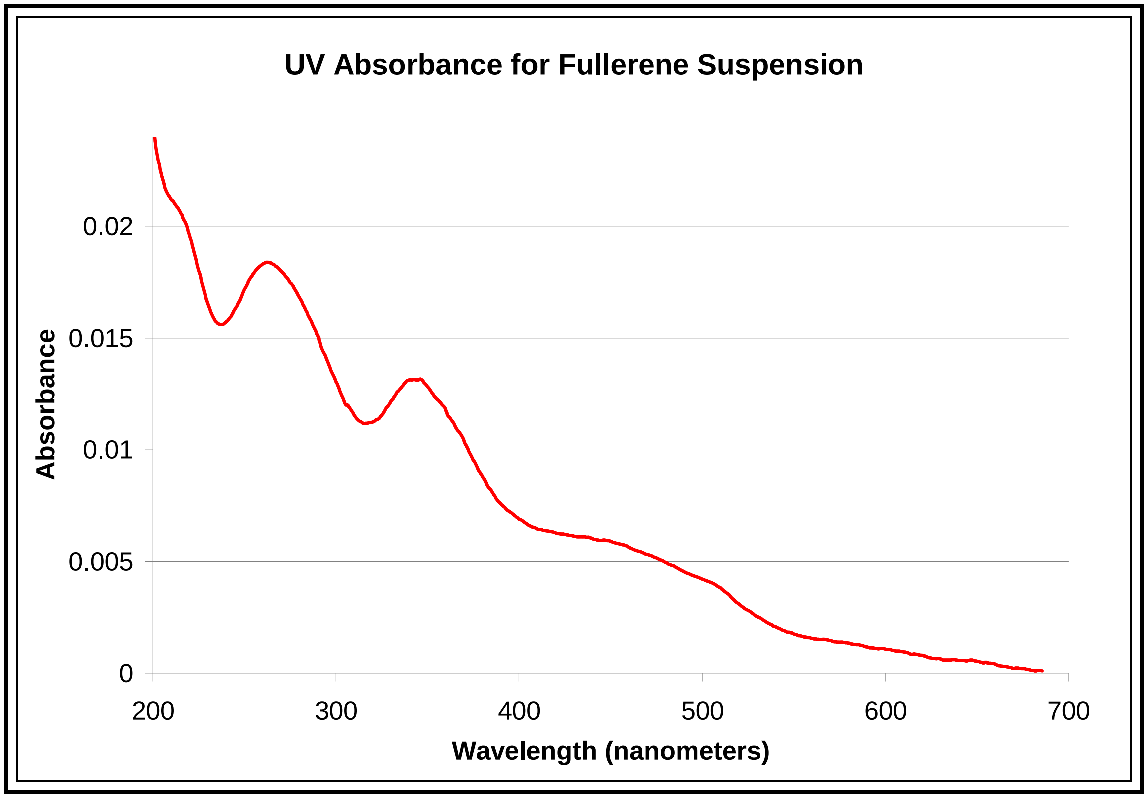

Figure 5 – Typical UV absorbance pattern for C_{60} fullerenes. Sample tested had a fullerene concentration of 1.436 mg/L

These findings were in line with those published by Brant, Lecoanet, and Wiesner (2005) and indicated that the suspension would remain stable over time, a condition that is necessary for this research. However, with repeated testing it was discovered that different batches had very different particle sizes. This is clearly evident in data found in the appendix and below in Table 3. Over the course of this research three stock suspensions of fullerenes were prepared and studied for average particle size. The average particle size of the suspension tested for stability was 202 ± 1.6 nm, while the other suspensions had effective diameters of 184 ± 6 nm and 273 ± 13 nm. Such a large variation in particle size was most likely due to the rotational speed of the stir bar since this was the least carefully controlled variable. Fullerene concentrations were kept within ± 0.01 g and mixing times within ± 2 days while, due to the use of different stir plates from different manufacturers, rotational velocity was difficult to account for.

Fullerene Particle Sizes	
Date	Effective Diameter (nanometers)
8/13/07	202
2/14/08	184
4/28/08	273

Table 3 – Fullerene particle size for various stock suspensions

Experience gathered while conducting this research indicated that there may be a certain particle size after which stability is compromised and should be studied further. However, stability was always ensured since particle size and UV absorbance were measured before conducting each column experiment.

Although more trials are necessary in order to arrive at definitive conclusions, it appears that particle size varies with pH. The suspension tested at pH 5 changed from an effective diameter of 184 ± 6 nm to 224 ± 28 nm, a size difference of 17.9%. Similarly the particles in the pH 9 suspension changed from an effective diameter of 273 ± 13 nm to 290 ± 32 nm, a size difference of 6.2 %. This is illustrated below in Figure 6.

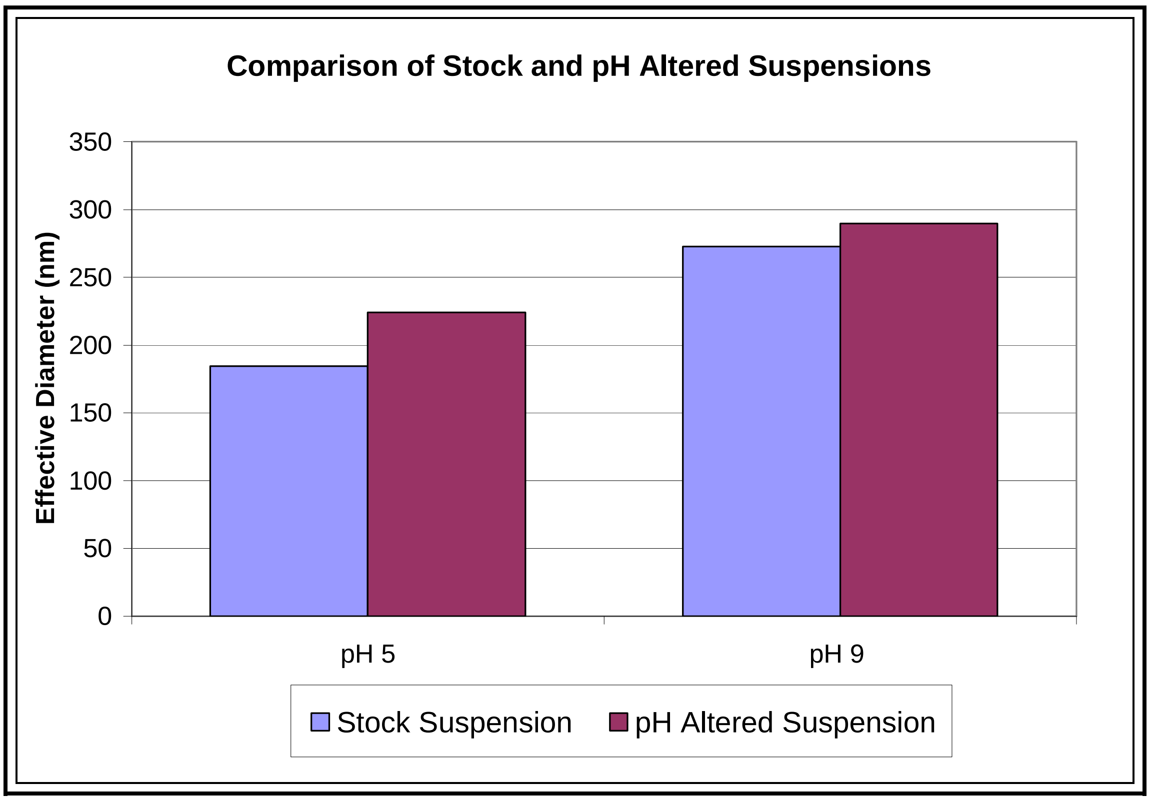

Figure 6 – Change in C_{60} fullerene particle size with pH

Two important variables should be noted however. First, the stock suspensions and pH altered suspensions were tested for particle size on different dates as noted in the Appendix. However, according to the stability study (Figure 4), any particle size variation over this time was likely negligible. Second, some of this variation of size is invariably due to the difference in ionic strength between the stock suspension and that of the pH altered suspension. As indicated in Figure 7, it can be expected that as ionic strength increases so will particle size. Size variation can still be compared against pH since the ionic strength of both pH altered suspensions was 0.01 M.

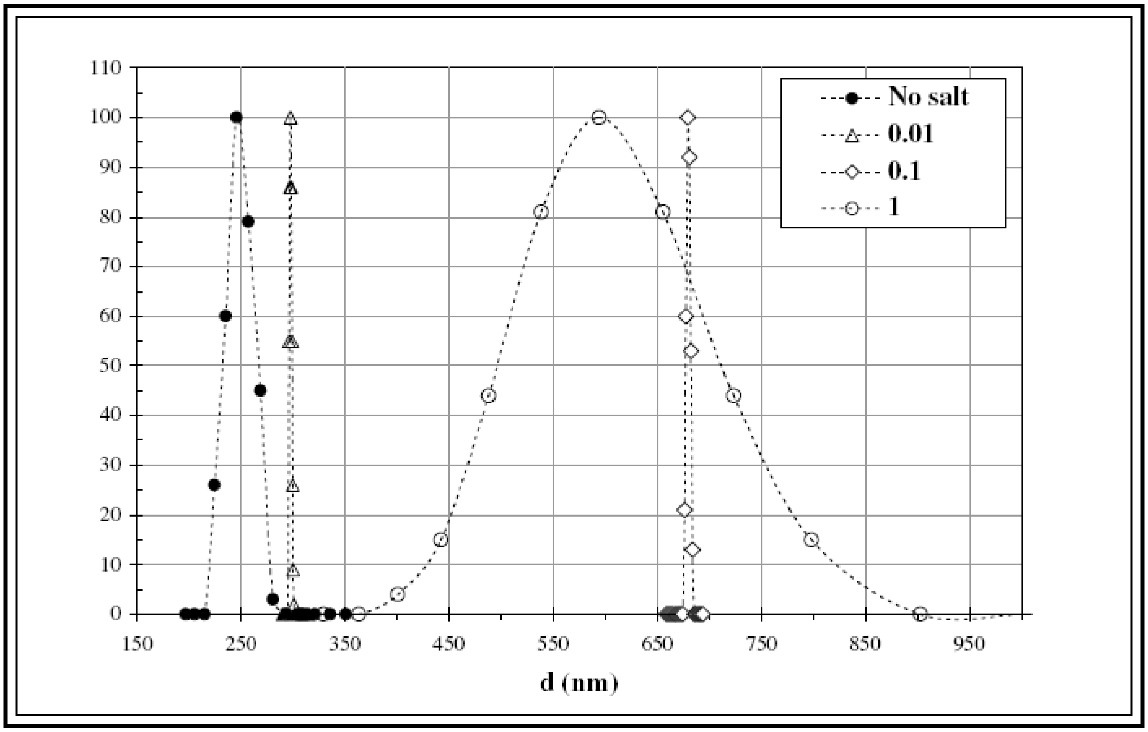

Figure 7 – Variation of C_{60} fullerene particle sizes in suspension with ionic strength. Taken from Brant, Lecoanet, and Wiesner (2005)

3.2 Column Experiments

A column experiment was performed using an influent solution of 100 mg/L KBr as Br. The breakthrough curve was fit using STANMOD (version 2.2) – CXTFIT2 (Simunek et al. 1999) computer software and results are included below in Table 4 and Figure 8. Modeling indicated that the dispersion coefficient was 0.25 cm^2/min and the linear velocity (v_s) was 2.0 cm/min. The dispersion coefficient is a measure of the dispersion of an advective fluid. From these values, a column Peclet number of 79 was calculated. The Peclet number is a dimensionless number relating the rate of advective flow to diffusive flow. Since the Peclet number was much greater than 1, flow through the column was advective (Chin 2000). Based on these values, the porosity and pore volume of the column were calculated to be 0.4 and 20 mL, respectively. After the hydraulic properties of the column were characterized, fresh columns were packed and experiments conducted using fullerene suspensions at pH 5 and pH 9 as discussed in the Column Experiment Methodology section above. Breakthrough curves for all three experiments are shown below in Figure 9.

Fitting Results	
Dispersion Coefficient	0.25 cm^2/min
v_s	2.0 cm/min
Porosity	0.4
Peclet Number	79 (v_s*L/D)

Table 4 – KBr curve fitting results using STANMOD v.2.2 (Simunek et al. 1999)

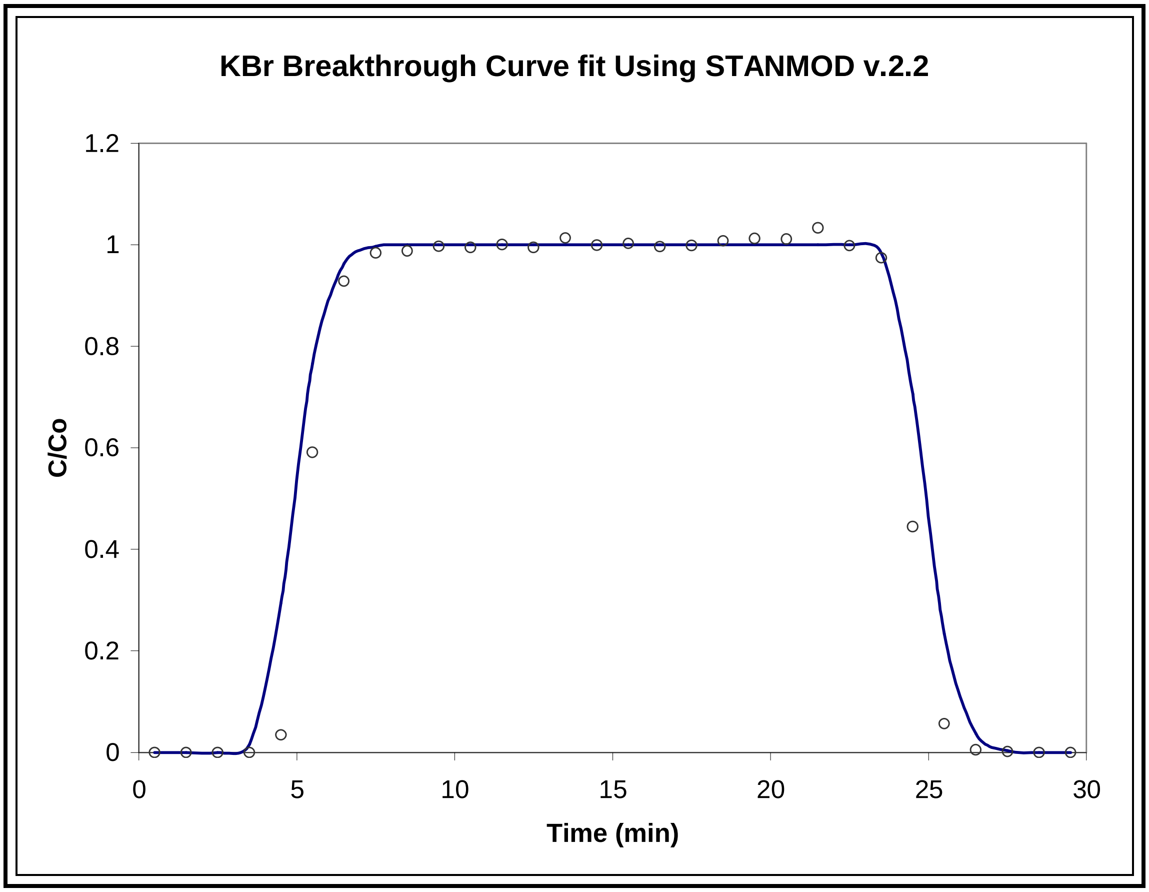

Figure 8 – KBr breakthrough curve fit using STANMOD v.2.2 (Simunek et al. 1999)

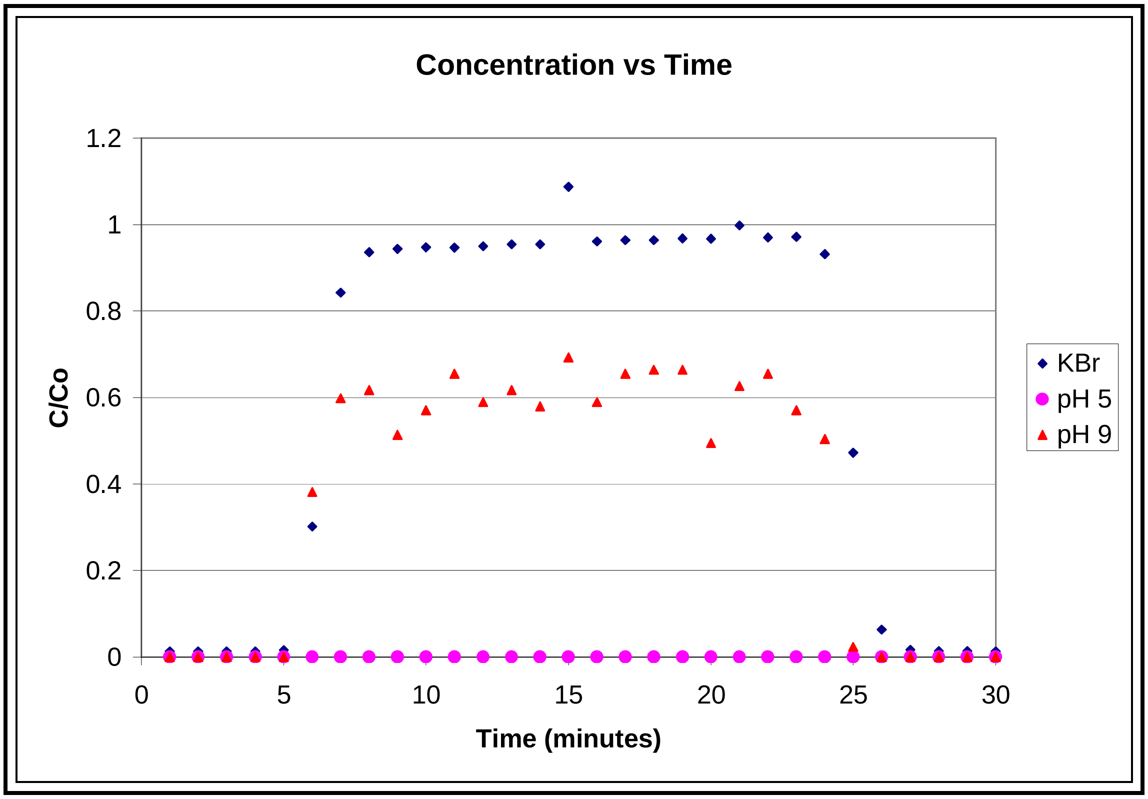

Figure 9 – Breakthrough curves for various column experiments

There was no breakthrough experienced in the pH 5 column experiment and a breakthrough of approximately 60 percent was measured in the pH 9 column. These results were most likely due to the difference in the electric potential on the surface of fullerene and sand particles at pH 5 and pH 9. As described by McDowell-Boyer (1992), fine particles surrounded by water form an electrical double-layer as ions opposite in charge from the particle align themselves around the particle. This results in a small electrical potential difference across the diffuse double-layer.

The electrophoretic movement of the charged particles due to an applied electric field can be then measured. In turn, the electophoretic mobility of a particle is often expressed as zeta potential (ζ). Zeta potential is a way of portraying, "the electrostatic potential at the plane of shear," as described by McDowell-Boyer et al. (1986). Sand particles and C_{60} fullerenes at pH 5 have a lower zeta potential than at pH 9 as indicated in Figures 10 and 11 below.

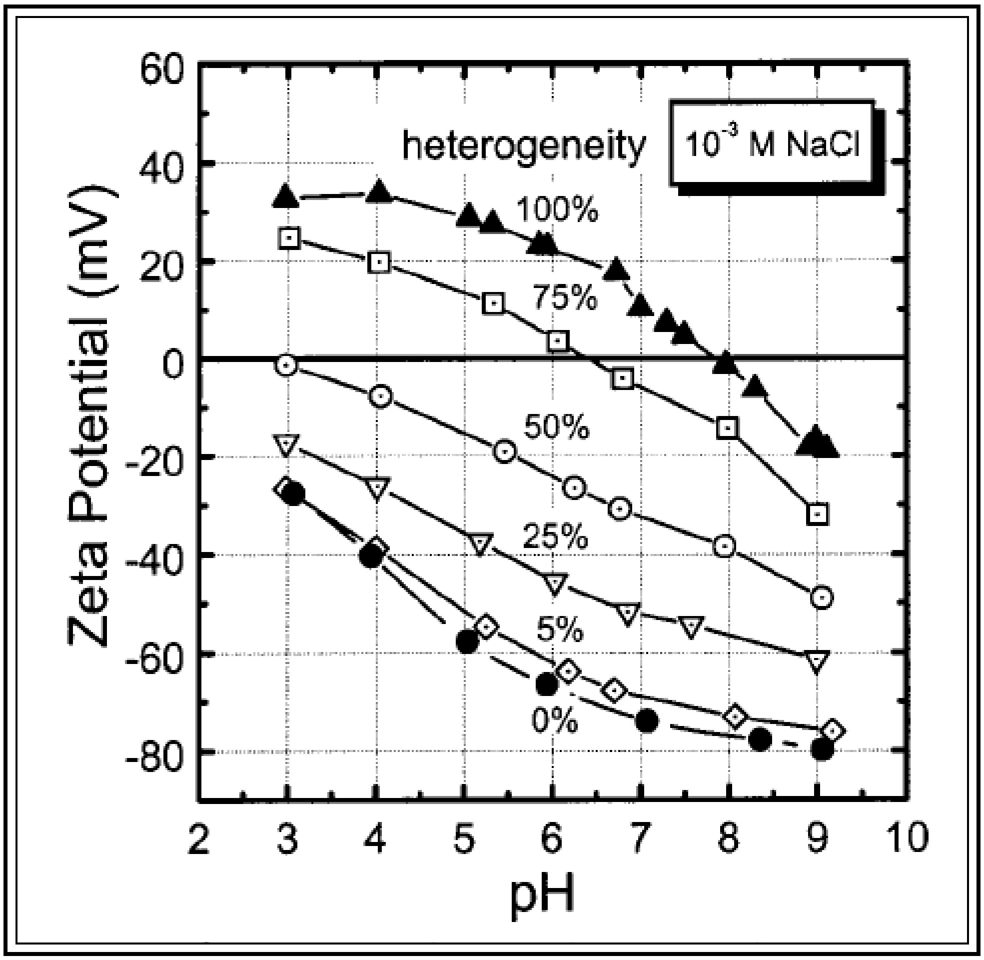

Figure 10 – Zeta potential as a function of solution pH for clean sand grains (filled circles), aminosilane modified sand grains (filled triangles), and various mixtures of aminosilane-modified and clean sand grains (open symbols). Taken from Elimelech et al. (2000)

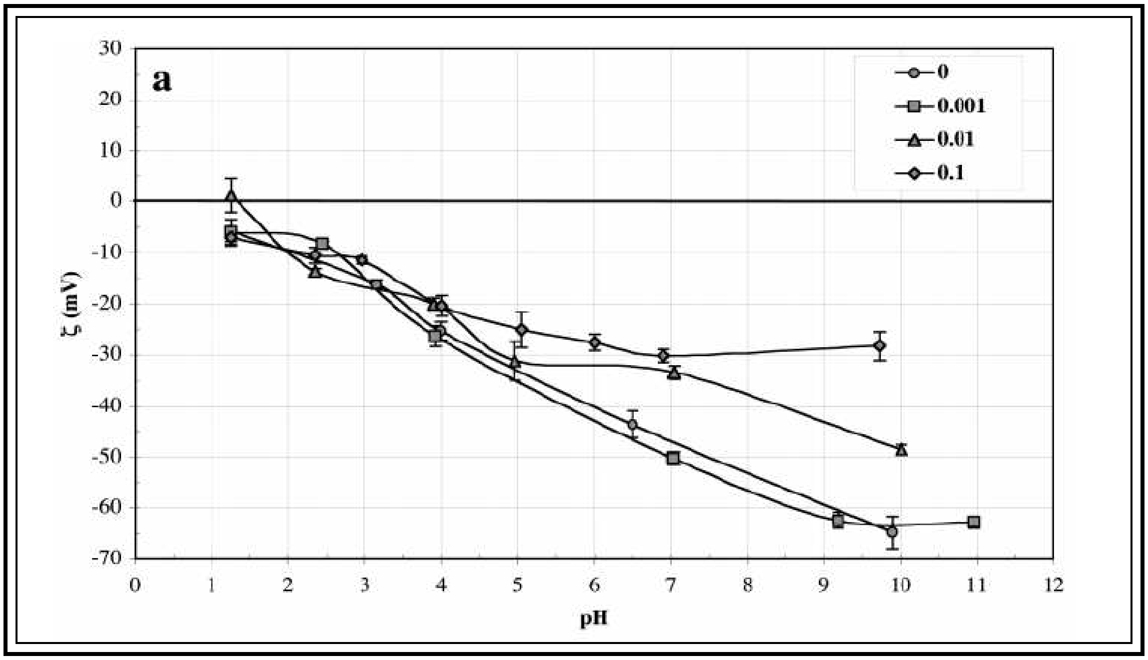

Figure 11 – Zeta potential vs. pH for C_{60} fullerenes in suspension at various ionic strengths. Taken from Brant et al. (2005)

Lower electric potential will increase the ability of the particles to become close enough to attach. This was indicated in the particle sizing experiments as well since fullerene clusters were larger at pH 5 than in the stock solution or at pH 9. Retention of particles in the sand column is controlled by, "physical-chemical filtration," (McDowell-Boyer et al. 1986) which supports the theory of why fullerenes were retained in the column at pH 5 and not at pH 9.

3.3 Reproduction Experiments

A summary of Encytraeid reproduction is shown below in Table 5. Reproduction was reduced in Sassafras soil compared to the Webster reference soil, but was not different between fullerene treatments. Reproduction in fullerene treatments was not different from reproduction in Sassafras soil controls (347±33) in a previous study, suggesting no effects of fullerenes on reproduction. Additionally, no mortality of adult worms was observed in any of the treatments suggesting no acute toxic effects of fullerenes on enchytraeids.

Mass C_{60}	0 mg	20 mg	200 mg
Soil Type	Webster	Sassafras	Sassafras
Test Vessel	Juvenile Worms Removed After 4 Weeks		
1	430	320	350
2	360	370	290
3	405	320	320
4	390	310	340
Average	**396^{a}**	**330^{b}**	**325^{b}**

Table 5 – Results of *Enchytraeus crypticus* reproduction test. Means with a common superscript are not significantly different (α=0.05, Tukey's Honestly Significant Difference)

CHAPTER 4

CONCLUSION

The aggregation and transport of colloidal particles are affected by many variables. This research has tested the effect of pH on C_{60} fullerenes in these regards. Apparently the difference in electrical potential across the diffuse double layer is affected by pH. This in turn causes a large variation in the ability of fullerene particles to aggregate to each other and attach to sand particles. While more examination is necessary, this research supports the findings of Brandt et al. (2005, 2006), McDowell-Boyer et al. (1986), and others. Many studies have analyzed the effect of pH and ionic strength on fullerene aggregation and deposition but no research could be found analyzing the transport of these particles through soil or sand. This research has shown that at pH levels likely to be found in the environment, fullerene particles will readily transport through sand. This also means that sand filters used at most water treatment plants will be unable to produce a significant reduction of fullerene concentrations in the water supply. However, at least some deposition is likely to be found in the terrestrial environment since at pH 9 40% of the particles were retained and 100% retained at pH 5. For this reason studies of the ecotoxicity of C_{60} fullerenes in the terrestrial environment appear necessary.

Reproduction tests performed on *Enchytraeus crypticus* worms in sandy soil found no effect on the mortality of adult worms or a significant effect on reproduction in sandy soil at concentrations of up to 1% by mass. A 1% concentration by mass is a much higher level than likely to be experienced in the terrestrial environment or underground aquifers. This is largely due to the extremely hydrophobic nature of these particles. Fullerenes are unlikely to attain very high concentrations in water in the natural environment. Accordingly, this research lends credence to the belief that these particles have no ecotoxicological effects. However, much more research needs to be done in this area.

APPENDIX

Fullerene Particle Size Over Time	
Date	**Effective Diameter (nanometers)**
8/6	200.0
8/13	202.1
8/20	203.2

Table 6 – Fullerene particle size over time

2/14 Size Characteristics For Stock Fullerene Suspension		
Sample	**Effective Diameter (nanometers)**	**Polydispersity**
1	190.6	0.216
2	194.0	0.225
3	186.2	0.189
4	179.2	0.235
5	179.6	0.244
6	177.3	0.247
7	189.5	0.245
8	174.5	0.222
9	189.0	0.284
Average	184.4	0.234

Table 7 – 2/14 Size characteristics for stock fullerene suspension

3/16 Size Characteristics for pH 5 Suspension		
Sample	**Effective Diameter (nanometers)**	**Polydispersity**
1	266.9	0.005
2	205.7	0.299
3	252.3	0.262
4	212.6	0.342
5	213.4	0.297
6	226.2	0.293
7	238.8	0.334
8	177.9	0.321
Average	224.0	0.291

Table 8 - 3/16 Size characteristics for pH 5 suspension

4/28 Size Characteristics For Stock Fullerene Suspension		
Sample	**Effective Diameter (nanometers)**	**Polydispersity**
1	269.7	0.283
2	271.8	0.255
3	285.9	0.295
4	263.5	0.316
5	270.1	0.280
6	254.3	0.259
7	293.7	0.283
Average	272.7	0.282

Table 9 - 4/28 Size characteristics for stock fullerene suspension

4/18 Size Characteristics For pH 9 Suspension		
Sample	**Effective Diameter (nanometers)**	**Polydispersity**
1	286.4	0.326
2	301.7	0.352
3	260.1	0.214
4	308.4	0.356
5	266.9	0.257
6	277.3	0.312
7	363.0	0.333
8	268.4	0.181
9	274.9	0.188
Average	289.7	0.280

Table 10 - 4/18 size characteristics for pH 9 suspension

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